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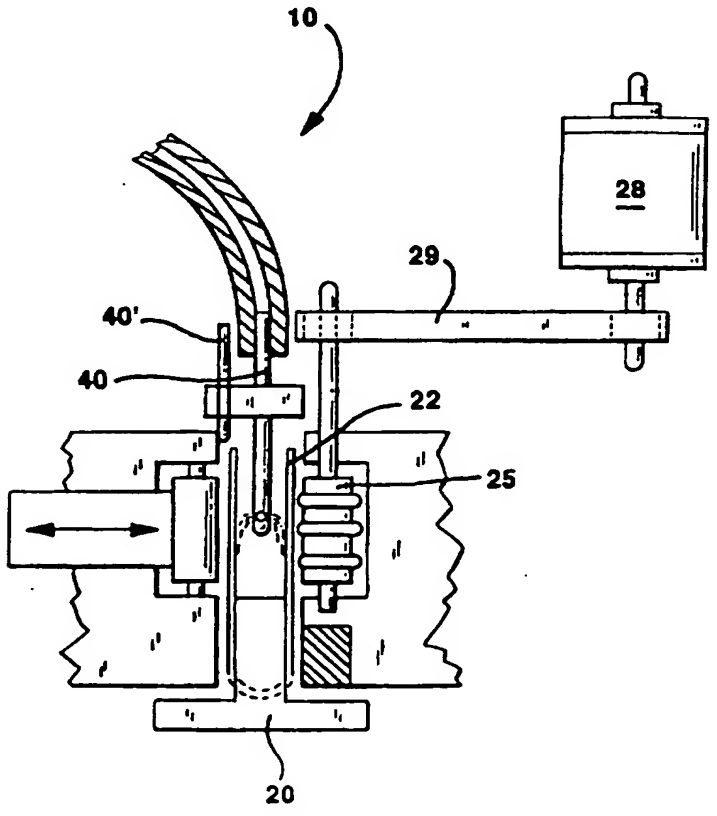
(54) Title: MEANS AND METHOD FOR WASHING REACTION VESSELS

(57) Abstract

A method and apparatus for effectively and reproducibly washing vessels in automated chemical analyzers is provided. A vessel washing station (10) having a driver (25) for rotating a vessel (22) about its axis and a probe (40) adapted t dispense a wash fluid into the vessel (22). The probe (40) includes an elongated tube which may have a closed bottom end and one or more ports arranged to dispense fluid downwardly and generally radially outwardly from the probe (40) to impinge on the inner wall of the vessel (22) as it is rotated by the driver (25). The probe (40) may also be adapted to aspirate fluid from the vessel (22) at the end of the wash cycle to rem ve waste fluid therefrom.



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MEANS AND METHOD FOR WASHING REACTION VESSELS

The present invention generally relates to devices for washing containers, and has particular utility in washing reaction vessels used in automated chemical analyzers and the like.

BACKGROUND OF THE INVENTION

Automated chemical analyzers have gained widespread acceptance in clinical and laboratory settings. In the medical field, the results of chemical testing can be very important in diagnosing an ailment, testing for the presence of specific substances and the like. Since important decisions can be made based on the results of the tests being performed, accuracy and precision are important in a reliable chemical analyzer.

Several factors determine how accurate and precise a test performed on an automated analyzer will be. Some of these factors are related to the instrument and the way a particular assay is performed by the instrument. Some of the factors involve the reagents used in the assay and the format of the assay. The effectiveness of a washing step performed by an automated analyzer during a test is one factor that is important. Particularly, in assay methods where a labeled reagent is used to detect the presence or amount of analyte in the sample is used and it is necessary to separate substances to which the labeled reagent is bound from other substances in the reaction solution.

Various assays have been described in the literature in which such labeled reagents are used. One type of assay often performed on automated analyzers involves the use of a solid phase, such as the walls of a reaction vessel, beads, particles, and the like and during the performance of the assay, the labeled reagent which is initially in a liquid phase, becomes bound to the solid phase in an amount that is related to the amount of analyte present in the sample.

In such assays, a predetermined quantity of a sample suspected of containing the analyte of interest, such as a volume of a patient's serum, is combined in a disposable reaction vessel in one or more steps with the

components necessary for conducting a specified test. One of the components of the t st may be a predetermined quantity of magnetically attractable particles on which an antibody that binds specifically with the analyte has been directly or indirectly immobilized. Another component is typically a second antibody that binds specifically with the analyte that has been labeled with a substance such as an enzyme, radioactive isotope, or the like that can produce an optically detectable signal. In such an assay, any analyte present in the sample will bind to the antibodies immobilized on the particles. Then the labeled antibodies will bind to the bound analyte. The amount of labeled antibodies used in such an assay is chosen so that it will exceed the amount of analyte likely to be present in the sample to insure that all bound analyte reacts with labeled analyte so that it can be detected. Then the solid phase containing the bound analyte is separated from the sample solution and the amount of label present on the solid phase is determined. The detection of label present on the solid phase typically requires the addition of a reagent that will react with the label to produce an optically detectable signal. It is therefore desirable to wash the reaction vessel and the solid phase with a wash solution to ensure that all unbound labeled reagent is removed. If any unbound labeled reagent remains in the reaction vessel, the label will be detected during the detection step of the assay and the results of the assay will be inaccurate.

Some commercial automated analyzers use a luminescent substrate that reacts with an enzyme label to generate an optically detectable signal. In such systems, the solid phase contained in the reaction vessel is washed with a wash solution to remove all but the solid phase and the labeled reagent bound to the solid phase. A luminescent substrate is then added that reacts with the enzyme label to generate a luminescent signal. The brightness of the signal is related to the amount of the bound labeled reagent and by measuring the intensity of this signal with a luminometer or the like, the analyzer is able to calculate to determine the quantity of analyte in the original patient sample.

The luminescent signal detected by the analyzer is therefore directly related to the quantity of the analyte in the reaction vessel in the assay described above. (In other types of assays, the relationship between the luminescence and the quantity of analyte may be different, but these two values will still be related in some way.) If the wash step is not very efficient, a significant quantity of unbound labeled reagent will remain in the vessel. This unbound labeled reagent will contribute to the signal detected by the luminometer, increasing the apparent quantity of analyte in the patient sample. This can obviously lead to inconsistent results if not properly monitored. Furthermore, if the quality of the washing varies from one vessel to the next, which is not uncommon, this will introduce a certain amount of statistical variation from one test to the next even for two identical samples.

However, the higher the noise in the data generated by the system, the higher the threshold of detectability of the system will be, that is, the higher the concentration of analyte in the sample will have to be in order for the intensity of signal to be considered distinguishable from noise. For some assays in which it is desirable to detect very low concentrations of the analyte in the sample, the noise level of a system which does not wash samples very well can be almost as high as the reagents detected by the luminometer in a properly run sample. This can easily lead to inconclusive or erroneous results. As a result, the benefit of some of the significant advancements in luminometers and the chemistry of luminescence can not be fully realized in such automated analyzers because the greater accuracy and precision afforded by these advances are overwhelmed by statistical variations in results attributable to inconsistent or ineffective washing of the reaction vessels. If one were able to more thoroughly and more consistently wash reaction vessels in an automated analyzer, the net sensitivity of the system could be greatly increased.

SUMMARY OF THE INVENTION

The present invention provides a device and method for effectively and reproducibly washing vessels, such as reaction vessels for use in automated chemical analyzers and the like. In accordance with a first embodiment of the

invention, a vessel washing station comprises a vessel support having a driver for rotating the vessel generally about its axis and a probe adapted to dispens a wash fluid int the vessel.

One embodiment of the probe includes an elongate tube which may have a closed bottom end and one or more ports arranged to dispense fluid downwardly and generally radially outwardly from the probe to impinge on the inner wall of the vessel as it is rotated by the driver. In one particularly preferred embodiment, the probe comprises an elongate tubular member having a hollow interior and a closed end, with one port in fluid communication with the interior of the tubular member to dispense fluid both downwardly and radially outwardly as the vessels is turned about its axis. The probe may also be adapted to aspirate fluid from the vessel at the end of the wash cycle to remove waste fluid therefrom.

Another embodiment of the invention provides a method for washing reaction vessels and the like. In accordance with this method, a washing station capable of supporting and rotating a vessel is provided with a probe adapted to dispense fluid downwardly and generally radially outwardly onto the walls of the vessel. The probe is lowered into the vessel to a point positioned above the maximum fluid level of the vessel and the vessel is rotated generally about its axis. As the vessel is rotated, the probe dispenses wash fluid to impinge on the walls of the vessel at or above the maximum fluid level of the vessel and the wash fluid is allowed to flow generally down the walls of the vessel and into contact with the vessel contents. Once a predetermined quantity of the fluid has been dispensed into the vessel through the probe, the waste contents of the vessel can be removed, such as by aspiration.

If so desired, the vessel can continue to rotate during such aspiration. The same probe used to dispense wash fluid into the vessel can be used for the aspiration of the waste contents or, in an alternative embodiment, a separate aspiration probe can be used to remove the waste fluids from the vessel. If further washing is needed, this wash process can be repeated any number of times until satisfactory washing has been accomplished.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 is a schematic illustration of a wash station in accordanc with the present invention;

Figures 2 A and B are an end view and a cross sectional view, respectively, of one embodiment of a probe in accordance with the invention;

Figures 3 A and B are an end view and a cross sectional view, respectively, of another embodiment of a probe in accordance with the invention; and

Figure 4 is a schematic cross sectional views of additional embodiments of probes in accordance with the invention.

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

Figure 1 shows, in schematic fashion, one embodiment of a wash station 10 in accordance with the invention. The wash station includes a vessel support 20 for holding vessels 22 received therein generally upright. Most reaction vessels take the general shape of a test tube, namely an elongate tube with a hollow interior and a closed bottom end. The precise shapes of the reaction vessels, including the height of the vessel and the shape of its bottom and wall or walls, can vary from one automated analyzer to the next. Obviously, the vessel support should be shaped to adequately support the vessels to be received therein. The embodiment in Figure 1, therefore, is merely intended to conceptually illustrate the vessel support and the actual shape and size of the vessel support used in any given analyzer will depend directly upon the shape and size of the vessels used in the analyzer.

The vessel support includes a driver 25 which is adapted to rotate the vessel generally about its axis. It is known in the art to spin vessels during washing, but the nature of the rotation and the reason for rotating the vessel varies from one wash protocol to another and the nature of the solid phase contained in the vessel. For example, in some industry-standard washing protocols, vessels are rotated relatively rapidly to urge a particulate solid phase outwardly against the vessel wall to permit less restricted access to the fluid in the vessel. In some other protocols, the vessels are rotated in alternate directions, switching between clockwise and counterclockwise

rotation about the vessel's axis, to promote more th rough mixing of the components of the vessel.

The capabilities of th driver in terms of speed, rotational direction and the like will need to be determined separately for different analyzer designs. In most cases, though, suitable drivers (e.g. electric motors 28 and belt drives 29) will be commercially available or can be readily designed with routine skill.

The connection between the driver and the vessel can be either direct or indirect. In a "direct" connection arrangement (as shown in Figure 1), the driver may include a surface which frictionally engages an outer surface of the vessel, such as the bottom or side of the vessel. In a more indirect connection, the vessel support may be rotatably mounted on a platform or directly mounted on a shaft attached to the driver. The driver can then spin the vessel support and the vessel support can translate its motion to the vessel held therein.

The vessel washing station of Figure 1 also includes at least one probe 40. In a single probe design, the same probe will typically be used to dispense wash fluid into the reaction vessel and aspirate the resultant waste fluid out of the vessel. As this can contaminate the probe and increase the risk of cross-contamination between samples in different vessels, it is preferred that there be a separate aspiration probe 40' disposed adjacent the vessel so that it can be positioned within the vessel to withdraw fluid.

The aspiration probe 40' is shown as being positioned immediately adjacent the washing probe 40 in Figure 1. In many analyzers, though, vessels are moved along a processing track. If the vessels are moved after the initial washing with the first probe 40, the aspiration probe 40' may be positioned over the next position the vessel will occupy to avoid any interference between the two probes.

Most probes commonly used for washing reaction vessels in analyzers and the like dispense a wash fluid directly downwardly into the vessel. A probe in accordance with the present invention, however, dispenses a wash fluid generally radially outwardly from the probe to impinge against the walls

of the vessel. The fluid is then allowed to flow down the sides of the vessel to wash any reactants clinging to the vessel walls down into the bottom of the vessel.

If so desired, the fluid can be directed at an angle substantially perpendicular to the axis of the probe, extending directly outwardly toward the vessel walls. In a preferred embodiment, though, the probe instead directs the fluid in a stream both generally radially outwardly and downwardly toward the vessel walls, as illustrated in Figure 1 by the droplets. (It is to be understood that the fluid will more likely flow out of the probe in a fashion closer to a continuous stream than in discrete droplets as shown; the drawing is just intended to schematically illustrate the direction of flow from the probe toward the vessel walls.) Several different probe tips designed to provide such a flow are described below in connection with Figures 2-4.

The probe 40 is desirably vertically actuatable to permit the operator (e.g. a controller of an automated analyzer) to carry the probe above the tops of the vessels, but lower the probe into the vessel for washing. This permits the vessels to be moved when the probe is not in use without hindrance from the probe.

The distance which the probe descends downwardly into the vessel will depend, at least in part on the direction in which fluid exits the probe, the volume of fluid to be contained in the vessel, and the rate at which the vessel is rotated by the driver. It is highly desirable to position the probe within the vessel so that the fluid from the probe will strike the vessel walls at least as high as, and preferably above, the maximum fluid level in the vessel. This will insure that the wash fluid will have an opportunity to wash off (or, at the very least, dilute) essentially all of the fluid clinging to the vessel walls.

It is important, though, that the probe not be positioned too high within the vessel. In particular, care should be taken to avoid splashing of the wash fluid too high up the vessel wall, or even out of the vessel, when it is being dispensed. For a given position of the probe within the vessel, the most important factors affecting the tendency of fluid to splash too high within the vessel are the orientation of the ports in the probe (discussed in some detail

below), the flow rate of the fluid through the ports (which will tend to control the force with which the fluid strikes the vessel walls), and the spinning rate of the vessel. Generally, too vigorous splashing is more likely when the probe's ports are oriented to direct fluid in a direction farther away from a vertically downward direction, at faster fluid flow rates, and/or when the vessel is spinning more quickly.

By adjusting the position of the probe within the vessel, though, one can achieve the desired flow rates, vessel rotation, etc. yet minimize the risk of splashing losses. For example, the probe can direct fluid directly radially outwardly (or even slightly upwardly from the probe tip), fluid can be pumped through the ports of the probe at a high flow rate, and the vessel can be rapidly rotated about its axis if the distance between the top of the vessel and the top level of fluid within the vessel is greater. This is because the probe can be positioned lower within the vessel (with respect to the top of the vessel), making it more difficult for fluid to splash upwardly over the top of the vessel.

If the vessel's dimensions are fixed, it may be necessary to position the probe at a location where there is some risk of contacting the fluid within the vessel to achieve the necessary flow rates and vessel rotation speeds to sufficiently wash the vessel yet still avoid fluid loss due to splashing. If this is necessary, the probe tip can be made of, or at least coated with, a hydrophobic material, e.g. polytetrafluoroethylene (Teflon). This will help minimize the amount of fluid clinging to the probe tip and, hence, the risks of significant cross-contamination of samples.

The volume of fluid added to a reaction vessel within a particular analyzer typically will fall within a fairly narrow range, with some relatively minor variations between specific types of assays due to the reagents used, the volume of the sample needed, etc. When the vessel is stationary, the fluid in the vessel will therefore generally have a fairly predictable level which will not exceed a predetermined height.

However, when the vessel is rotated by the driver 25, centrifugal force will tend to urge more of the fluid against the walls of the vessel and the level

f the fluid along the walls will rise. For a given driver operating in a prescribed fashion and a known range of fluid properties, the maximum fluid level, i.e. the maximum height of fluid along the vessel walls, should be readily determined. By directing the wash fluid outwardly onto the vessel walls at a point at least as high as this maximum fluid level, a much more uniform and efficacious washing will be achieved than if the fluid is directed straight downwardly, as is currently done.

Figures 2-4 illustrate several different embodiments of probes in accordance with the present invention. (While any one of these probes can be used in the vessel washing station of Figure 1 wherein the probe is designated by the reference numeral 40, each of these probes will bear separate reference numbers for the sake of clarity in the discussion below.) Each of these probes have some structure in common, namely an elongate, generally tubular body 42 having a wall 44 defining a hollow interior 46 through which fluid passes. The probes differ primarily in the structure at the lower end of the probe and the number, shape and arrangements of the ports carried by the probe.

Turning first to Figures 2 A and B, the probe 50 shown therein has a sealed end cap 52, which may define a generally hemispherical dome shape, as best seen in Figure 2B. A plurality of slots 54 are formed in the probe, each slot defining a chord across the generally circular bottom view of the probe, as clearly shown in Figure 2A. The slots extend inwardly through the wall 44 and communicate fluid from the interior 46 of the probe outwardly toward the vessel walls. Although the slots could be deeper than as shown in Figure 2B, the slots in Figure 2B intersect the hollow interior of the tube only for a short distance along the middle of their length, creating a relatively small passageway 56 between the interior of the probe and the slots.

The illustrated embodiment shows three slots 54 arranged generally equiangularly about the circumference of the probe. Such an equiangular arrangement has the advantage of providing more uniform fluid distribution over the vessel wall, but the faster the vessel is rotated by the driver 25, the less likely it is that a lack of uniform distribution from the probe tip will cause

a problem in washing. It should also be understood that there may be more or fewer slots, such as a single slot oriented at the desired angle with respect to the axis of the probe (as shown in the preferred embodiment of Figure 4) if the driver is to rotate the vessel rapidly enough to ensure uniform fluid distribution on the vessel walls from one port.

As fluid exits through the passageway 56, it will tend to fan out along the slot somewhat, with the shape and orientation of the slot, together with the rate of fluid delivery, generally dictating the width and angle of the stream of fluid delivered from each of these ports. The angle at which the probe directs the flow of fluid can thus be controlled to a large extent by the angle at which the slots 54 are oriented with respect to the axis of the probe. In the embodiment shown, the slots are oriented at an angle of about 80° with respect to the axis of the probe. It should be understood, though, that this angle can be varied substantially, with an angle in the range of about 45-90°, and more desirably about 60-75°, being preferred for most applications.

Figures 3A and 3B illustrate an alternative probe 60 for use in the present invention. In this embodiment, the probe has a series of generally tubular ports 64 extending at an angle through a generally circular block 62 which effectively seals the end of the probe. The embodiment illustrated in Figures 3A and 3B employs four ports rather than the three ports shown in the probe 50 in Figures 2A and 2B, illustrating the point made above that the number of ports can be varied. This probe 60, like the probe 50, can have more or fewer ports, as desired.

Although the ports 64 can be arranged about the probe as desired, such as by orienting all of the ports toward one side of the probe, the ports are desirably spaced substantially equiangularly about the circumference of the probe. In Figures 3A and 3B, wherein four ports are used, the ports are therefore optimally spaced about 90° from one another. The angle of the ports with respect to the axis of the probe is shown as being about 60°, but this angle can also be varied between about 45° and about 90°, with an angle of about 60° to about 75° being preferred.

Figure 4 illustrates another alternative embodiment of a probe of the invention. Whereas the probes 50, 60 of Figures 3 and 4 employ a plurality of ports arranged generally equiangularly about the circumference of the probe, the probe 70 of Figure 4 uses a single port 74. The multi-port probes 50, 60 have the advantage of more uniformly distributing fluid from the probes onto the vessel walls, but at higher rotational speeds of the vessel this becomes less important, as noted above. As mentioned above, though, if the vessel is rotated rapidly enough, a single port design as illustrated in Figure 4 can instead be used.

The single port 74 of the probe 70 of Figure 4 is desirably oriented at an angle with respect to the axis of the probe of between about 45° and about 90°, with an angle of about 60° to about 75° being preferred. In the illustrated embodiment, this angle is about 60°. The probe 70 can be machined out of an integral piece of material, such as a stainless steel rod or tube.

In the preferred embodiment shown in Figure 4, though, the probe 70 is formed from an elongate tube 42, which may be formed of stainless steel, for example, and a separately formed plug 75 which serves to effectively seal the end of the probe and direct fluid through the port 74. The plug may be formed in any desired shape from any suitable material. The plug 75 of Figure 4 employs a rounded button 76 and a generally tubular projection 77 which extends into and is firmly received within the hollow interior 46 of the tube. In order to minimize cross-contamination of samples if the probe does contact fluid in a vessel, this probe tip may be formed of a hydrophobic polymer such as Teflon. It should be understood that this tube/plug structure is only illustrated in connection with tis single-port design, but the manufacturinng ease and other advantages it affords could be equally effective in probes having multiple ports.

Such a single port design has the advantage that, for a given total flow rate of fluid through the probe, the fluid will xit the single port with more force than if the fluid were distributed through several ports having a larger total cross secti nal area. The total volume of fluid which is to be dispensed

into the vessel is usually somewhat limited. By using a single port, for the same fluid flow rate and dispense time (i.e. for the same total fluid volume), the force with which the fluid strikes the vessel walls can be increased. It is believed that striking the vessel wall with more force can be beneficial (provided, of course, that the fluid does not splash out of the vessel) in that it will help wash off fluid clinging to the vessel walls. The probe 70 has been found to wash vessels very effectively at a flow rate of about 500μl per second while the vessel is rotated at about 1770-1800 revolutions per minute.

As noted above, the present invention also provides a particularly effective method for washing reaction vessels and the like with a fluid-dispensing probe. The method may be, and desirably is, carried out in a wash station 10 generally as outlined above, but any structure which provides the essential elements of the instant method could instead be used.

The present method desirably employs a wash station 10 capable of supporting and rotating a vessel which is provided with a probe adapted to dispense fluid downwardly and generally radially outwardly onto the walls of the vessel. The probe is lowered into the vessel to a point positioned above the maximum fluid level of the vessel and the vessel is rotated generally about its axis. As the vessel is rotated, the probe dispenses wash fluid to impinge on the walls of the vessel at or above the maximum fluid level of the vessel and the wash fluid is allowed to flow generally down the walls of the vessel and into contact with the vessel contents.

The fluid is desirably directed generally radially outwardly and downwardly from the probe, as noted above, with an angle of the fluid stream with respect to the axis of the probe desirably between about 45-90°, with an angle of about 60-90° being preferred, with 60°-75° being believed to be optimal. (It should be noted that the 30° angle shown in Fig. 4 is with respect to horizontal, not the generally vertical axis of the probe.)

Once a predetermined quantity of the fluid has been dispensed into the vessel through the probe, the waste contents of the vessel can be removed, such as by aspiration. If further washing is needed, the wash process of the

invention can be repeated any number of times until satisfactory washing has been accomplished.

By directing the fluid downwardly against the wall of the vessel, the wash fluid delivered by the probe will tend to cleanse the vessel walls of any fluid which may have splashed up there. Generally speaking, fluid striking the vessel wall with greater force will tend to more quickly cleanse the wall, but the total volume of fluid which should be dispensed into the vessel is limited and having fluid flow over the walls for too short a time will decrease the efficacy of the washing. Accordingly, one will need to balance these two factors (among others, such as the rotational speed of the vessel) to find an optimal flow rate of wash fluid through the probe. As noted above, the probe 70 of Figure 4 can be used at a flow rate of about 500μl per second and a vessel rotation rate of about 1770-1800 rpm with excellent results.

Rotating the wash vessel while dispensing the fluid through the probe also significantly improves washing. Rotating the vessel will change the orientation of the probe's ports with respect to the vessel wall, helping more evenly distribute the fluid over the wall. This same advantage can also be realized by rotating the probe while holding the vessel substantially stationary and this alternative can be practiced in carrying out an alternative method of the invention. However, as noted above, spinning the vessel also has the effect of driving the fluid up the walls of the vessel, frequently with some turbulence. This further cleans the walls of the vessel as the fluid level rises in the vessel. The orientation of the present probe's ports, combined with this spinning action, provides a particularly effective means for washing a vessel in accordance with the invention.

As noted above, the contents of the vessel after the wash fluid has been added can be aspirated out of the vessel for disposal. If so desired, the vessel can continue to rotate during such aspiration. The same probe used to dispense wash fluid into the vessel can be used for the aspiration of the waste contents. However, this would tend to contaminate the probe, requiring the probe itself be washed between each washing sequence.

Accordingly, it is preferred that a separate aspiration probe be provided for removing the waste fluids from the vessel.

In many automated analyzers, washing takes place while the vessels are on a conveyor belt or the like, moving past a stationary washing station (or washing stations) for further processing. In such designs, one can very readily have the probe 40 of the wash station positioned at one location along the vessels' path and position an independent aspiration probe adjacent the next location along that path. Although this design would require the addition of a second probe, certain aspects of the analyzer can be simplified if the wash probe need not itself be washed during normal operation and can instead be hooked to a dedicated wash fluid supply line (not shown) and the aspiration probe can be attached to dedicated vacuum and wash fluid lines.

The device and method of the present invention provide a means for consistently and thoroughly washing reaction vessels and the like. The results achieved with the present invention are surprisingly good and can enhance the sensitivity of an automated analyzer by reducing the statistical "noise" which can commonly result if the vessels are inconsistently or inadequately washed to remove unbound chemicals. This advantage can be particularly important if a reactant or reaction product in the vessel is more viscous or tends to cling to the solid phase or vessel walls. The following experimental examples illustrate the particular effectiveness of the present invention in connection with automated HIV and TSH assays, both of which tend to be particularly sensitive assays due to the relatively low-level signal produced in these assays.

Each of the tests carried out in Examples 1 and 2 employed an automated analyzer generally as described in PCT Application No. PCT/US93/04209, published as International Publication No. WO 93/22686. Such an analyzer is commercially available from Sanofi Diagnostics Pasteur Inc., USA under the trademark ACCESS. The wash buffers, reagents and solid phase magnetic particles used in these examples are all commercially available from Sanofi Diagnostics Pasteur as reagent packs or refill fluid supplies, unless otherwise noted. (For example, the HIV reagents were

supplied in a reagent pack purchased from Sanofi Diagnostics Pasteur under catalog no. 34000 and TSH tests were conducted using Sanofi Diagnostics Pasteur's reagent pack available under catalog number 33820.) Any operational details not set forth below can be readily ascertained from this commercially available analyzer and/or its associated manuals.

In the control runs, a standard probe was used. This standard probe comprised a simple tube with a flat, open end oriented generally vertically downwardly toward the bottom of the vessel being washed on each of the wash stations. In the experimental runs, a probe generally as described above in connection with Figure 4 was used, with virtually all other aspects of the system remaining the same. The only exception is that in the runs using the probe of the invention, the system began spinning the vessel shortly before dispensing the wash fluid rather than starting spinning after dispensing was completed, as was done with the standard probe. It is believed that the configuration of the analyzer and the specific reagents used in a particular assay are not critical and that qualitatively similar results would be obtained regardless of what overall analyzer design is used.

EXAMPLE 1 - HIV

Several patient samples were obtained, with each sample having been already determined to be HIV negative. The first 300 tests (Runs 1-6) were all carried out with a first patient sample, the next 23 tests (Run 7) was carried out with a different patient sample, and the final 23 tests (Run 8) was conducted with a third patient sample. For each run, the specific patient sample was diluted 1:11 with a standard wash buffer commercially available from Sanofi Diagnostics Pasteur.

To each reaction vessel was added 50 microliters of magnetic particles in a buffer solution (about 1 micron particle size at approximately 1 mg per μL), 50 μL of the diluted patient sample and 50 μL of antibody-alkaline phosphatase conjugate. The reaction mixture in the vessel was incubated at about 37 C for about 19.2 minutes.

The vessel was then transferred to a wash station. About 350 μL of the standard wash buffer solution was added to the reaction vessel, which

was incubated for about 12 seconds and placed in a magnetic field for about 144 seconds to separate the magnetic particles from the rest of the contents of the vessel. The contents of the reaction vessel were aspirated with a separate aspiration pr be. The tip of the aspiration probe was kept below the surface of the reaction mixture until the bottom of the vessel was reached to avoid disturbing the "pellet" of magnetic particles.

The vessel was then moved on to a second wash station and about 500 μL of the wash solution is added to the vessel. In this wash step the vessel is rotated at about 1770-1800 rpm in a first direction for about 4 seconds, rotated in the opposite direction for an additional 2 seconds, and then rotated in the first direction again for a final 2 seconds. The magnetic particles are separated in a magnetic field and the contents of the vessel are once again aspirated substantially as described above. The vessel is then moved on to a third wash station and essentially the same washing process carried out at the second wash station is carried out at the third wash station.

The reaction vessel then is moved on to a "read station". The reaction vessel is rotated at about 2500 rpm while about 200 μL of LumiPhos® brand 530 dioxetane chemiluminescent substrate is added. (LumiPhos® is commercially available from Lumigen Inc., USA.) The vessel is rotated at about 2500 rpm in a first direction for about 4 seconds, rotated at about the same speed in the opposite direction for 2 more seconds, and then rotated again in the first direction for a final 2 seconds. This reaction mixture is then incubated at about 37°C for about 6.4 minutes. The luminescence of the sample is then measured using a luminometer.

Six runs (identified below as runs 1-6) were conducted using a standard, bottom-dispense probe, while two runs (runs 7-8) were run using a probe substantially as shown and described in Figure 4. In each of runs 1-6, 50 samples were prepared and processed as outlined above and the numbers listed in Table 1 for each run reflect the combined results of these 50 tests. In each of runs 7 and 8, only 23 samples were prepared and processed. The numbers listed in Table 1 for each of these runs reflects the combined results of these 23 tests. (The numbers in Table 1 and Table 2 are set forth in

"relative light units" measured by the luminometer. The absolute values of these relative light units are not important; the *relative* values and the statistical relationships of these values are what is important here.)

Table 1

	Run 1	Run 2	Run 3	Run 4	Run 5	Run 6	Run 7	Run 8
# of Tests	50	50	50	50	50	50	23	23
Mean	17208	18435	16890	17511	17374	18876	11438	22574
Median	18015	15720	15793	15804	15554	16287	11329	22699
Minimum	14850	14524	15267	14748	14058	15349	11090	21330
Maximum	47459	80574	33539	44804	36643	50298	12832	23927
Range	32609	66060	16272	30058	22585	34947	1742	2597
St. Dev.	4930	9739	3400	5187	4496	6431	358	673
CV	28.7%	52.8%	20.1%	29.6%	25.9%	34.4%	3.1%	3.0%
Outlier Threshold	32029	31440	31586	31608	31107	32573	22858	45398
Outlier Count	1	2	1	2	1	2	0	0

The results of these tests clearly indicate that the statistical "noise" in the data collected is much lower for runs 7-8 than any of runs 1-6. Standard deviation in runs 1-6 range anywhere from about 20% to about 53% of the measured value, averaging about 32% of the mean value. In runs 7-8, though, the standard deviation was only about 3% of the mean.

Furthermore, in each of runs 1-6, at least one sample yielded a result which is at least twice the median value of the data. (Such a result is referred to in Table 1 as an "outlier", with the threshold for defining an "outlier" and the number of "outliers" being identified for each run.) There were no tests in either Run 7 or Run 8 which yielded results which were twice the median value for the run, i.e. there were no "outliers" for these runs. These results indicate that there is a lower likelihood of a significantly erroneous result for an individual sample when the samples are washed in accordance with the present invention as compared to a standard washing regimen.

Table 1 clearly indicates that the present invention can improve the results and reliability of automated testing. In particular, the data for samples washed using a probe in accordance with the present invention was much more consistent and had much less statistical "noise" than samples which were run essentially identically, but used a standard, bottom-dispense probe. Perhaps equally as importantly, the samples processed in accordance with the present invention exhibited essentially no "outlier" problems, enhancing the reliability of the system on a sample-by-sample basis, reducing the chance that the analyzer would yield an anomalous result for a given patient sample.

EXAMPLE 2 TSH

Assays for hTSH (human thyroid-stimulation hormone) were conducted to test the efficacy of the present invention. Four runs were conducted, each run comprising fifty tests. Two runs (identified as runs 1 and 2) were conducted using a standard, bottom-dispense probe, while the other two runs (identified as runs 3 and 4) were conducted using a side dispense probe generally as shown in Figures 2A and 2B.

To each reaction vessel was added about 45 μL of the same magnetic particle solution used in Example 1, 20 μL of protein-containing solution (about 1.5 mg/ml MIgG, 0.6 mg/ml GIgG, and about 0.1% BSA in a Tris buffered saline solution), 45 μL of goat anti-hTSH conjugated to alkaline phosphatase in a Tris buffered saline solution, and 100 μL of a standard sample. The standard sample used in these tests comprised a substantially hTSH-free commercially available buffer solution, available from Sanofi Diagnostics Pasteur. This reaction mixture was then incubated at about 37°C for about 33 minutes.

Substantially the same washing, incubation and luminescence detection steps outlined above in Example 1 were carried out on each of the TSH samples. The primary difference between these two runs is that in the first wash stage in Example 1, 350 μL of wash buffer was added at the first wash station; in the TSH runs of this example, the first wash step only utilized 275 μL. Otherwise, the tests were carried out substantially as outlined above in Example 1. The results of these test runs 1-4 are shown in Table 2.

Table 2

	Run 1	Run 2	Run 3	Run 4
No. of Tests	50	50	50	50
Mean	11,974	11,904	11,445	11,135
Median	111,665	11,721	11,399	11,105
Std. Dev.	2,103	744	230	204
CV	16.81%	6.25%	2.01%	1.83%
Outlier Threshold	14,581	14,651	14,249	13,881
Outlier Count	1	2	0	0

In this example, the definition of an "outlier" was reduced to any test value which exceeds the median by more than 25%, rather than twice the mean as in Table 1. Even under this reduced standard, the present tests yielded no "outliers" when run with a probe in accordance with the invention, while outliers were found in the data for standard probes. Furthermore, the standard deviations of the runs using the present invention were on the order of about 2% of the mean value; the tests run with a standard probe showed standard deviations as high as almost 17%.

The data from these tests are very promising. Particularly at lower levels of sample luminescence, it is believed that the present invention provides relatively consistent, marked improvement in washing the samples. While this improved washing may not always provide superior statistical conformity (e.g. lower standard deviations), it is believed that the present invention can be very helpful in reducing, or even eliminating, significantly anomalous results on individual samples, i.e. "outliers".

While a preferred embodiment of the present invention has been described, it should be understood that various changes, adaptations and modifications may be made therein without departing from the spirit of the invention.

WHAT IS CLAIMED IS:

1. A method of washing a reaction vessel, comprising:
   a. providing a washing station having a driver for rotating a reaction vessel received therein and a dispensing probe for dispensing a wash fluid;
   b. positioning a reaction vessel to be washed at the washing station;
   c. lowering the dispensing probe into the vessel;
   d. rotating the vessel with the driver and dispensing wash fluid from the dispensing probe downwardly and generally radially outwardly onto the walls of the rotating vessel; and
   e. removing a quantity of the dispensed wash fluid from the vessel.
2. The method of claim 1 wherein the reaction vessel has a maximum fluid level and the dispensing probe is lowered to a position no lower than the maximum fluid level.
3. The method of claim 2 wherein the maximum fluid level is determined by the maximum height on the vessel's wall which fluid in the vessel, including the wash fluid dispensed from the dispensing probe, will reach when the vessel is rotated.
4. The method of claim 1 wherein the wash fluid is removed from the vessel by aspiration.
5. The method of claim 4 wherein a separate aspiration probe is provided, the method further comprising withdrawing the dispensing probe from the vessel, lowering the aspiration probe into the vessel and aspirating wash fluid from the vessel through the aspiration probe.
6. The method of claim 4 wherein the fluid is removed from the vessel by aspirating it through the dispensing probe.
7. The method of claim 1 further comprising repeating steps d and e.
8. The method of claim 7 further comprising raising the dispensing probe from the vessel after each step of dispensing the wash fluid.
9. A washing station for washing a reaction vessel, comprising a driver for rotating a reaction vessel received therein and a dispensing probe in

fluid communication with a supply of wash fluid; the dispensing probe having an axis and comprising a generally tubular body having a hollow interior, a sealed bottom end and at least one port in communication with the hollow interior through which said wash fluid may pass, the port being angled to dispense fluid downwardly and generally radially outwardly from the probe adjacent its bottom end.

10. The washing station of claim 9 wherein the dispensing probe has an axis and the port is oriented at an angle of between about 45° and about 90° with respect to said axis.

11. The wash station of claim 10 wherein said angle is between about 60° and about 70°.

12. The wash station of claim 9 wherein the probe comprises a plurality of said ports spaced generally equiangularly about a circumference of the probe.

13. The wash station of claim 12 wherein four ports are used, each port being spaced from the next adjacent port by about 90°.

14. The wash station of claim 9 wherein the probe comprises a plurality of said ports, each port comprising a slot defining a chord across a lower portion of the probe.

15. The wash station of claim 14 wherein each slot intersects the hollow interior of the tube for a short distance along the middle of its length, said intersection defining a passageway between the hollow interior of the probe and the rest of the slot.

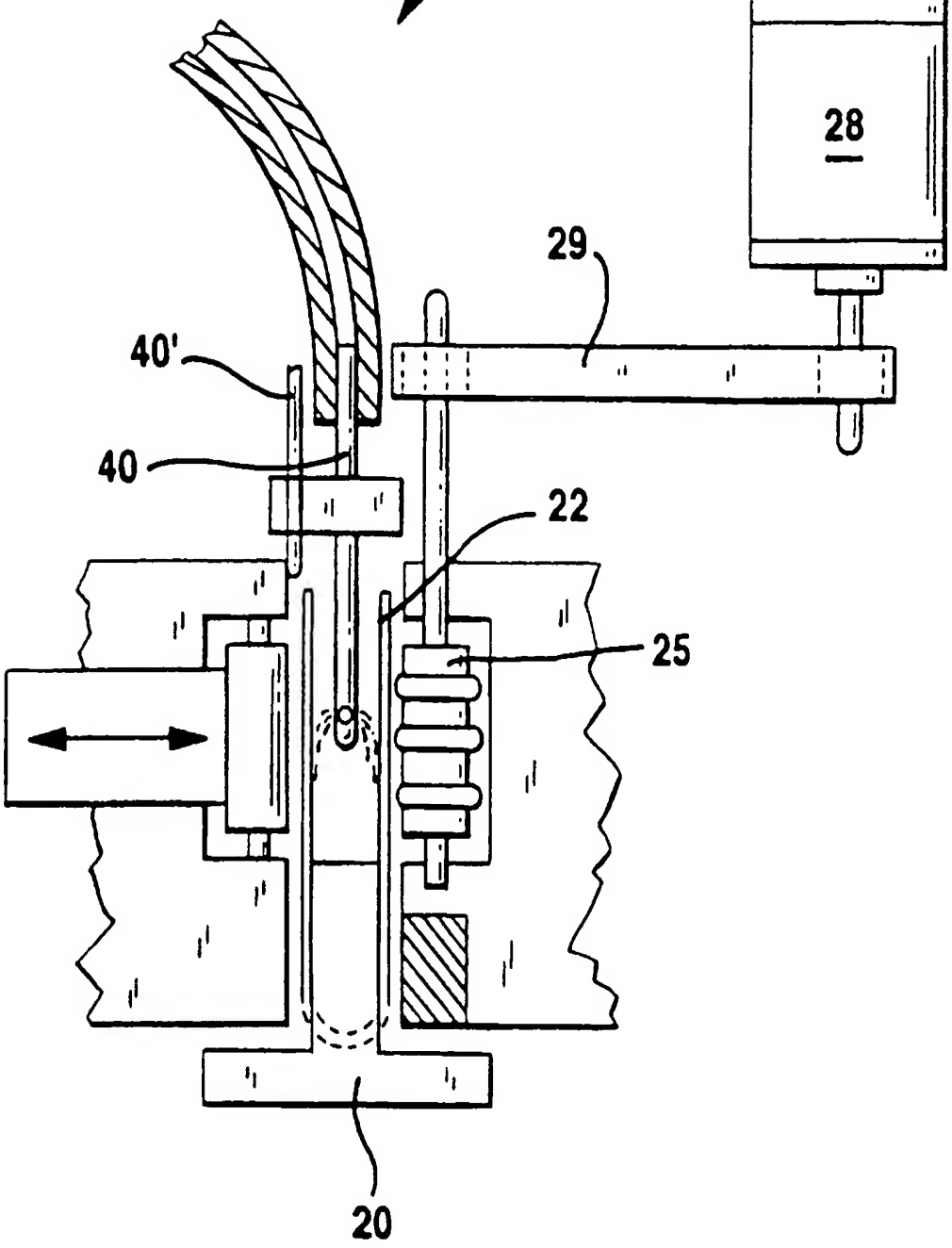

FIG. 1

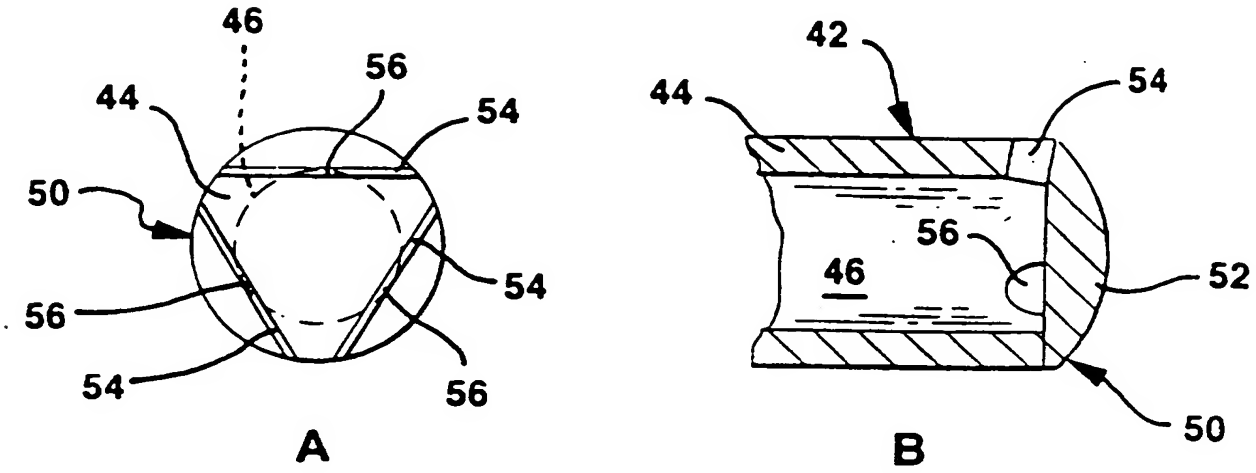

FIG. 2

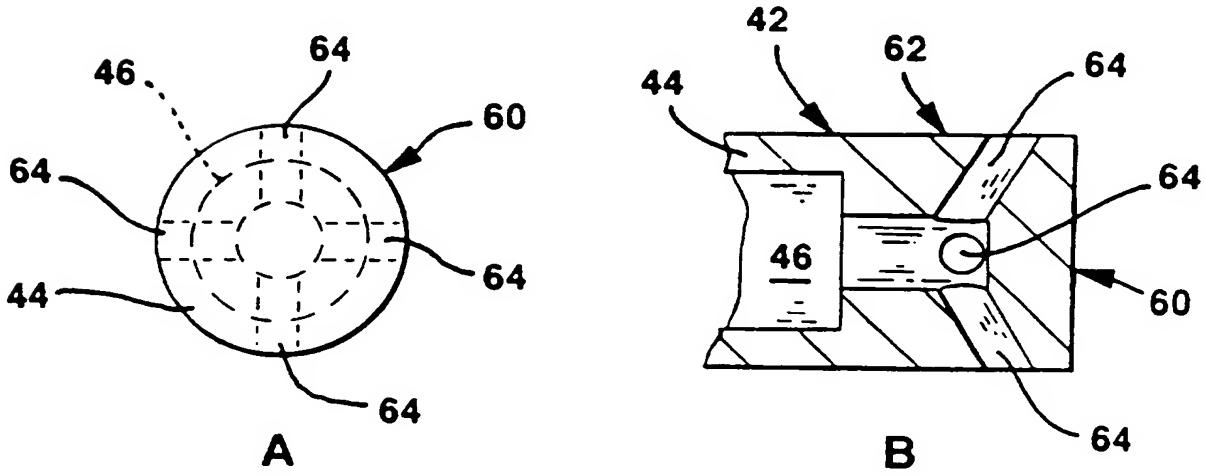

FIG.3

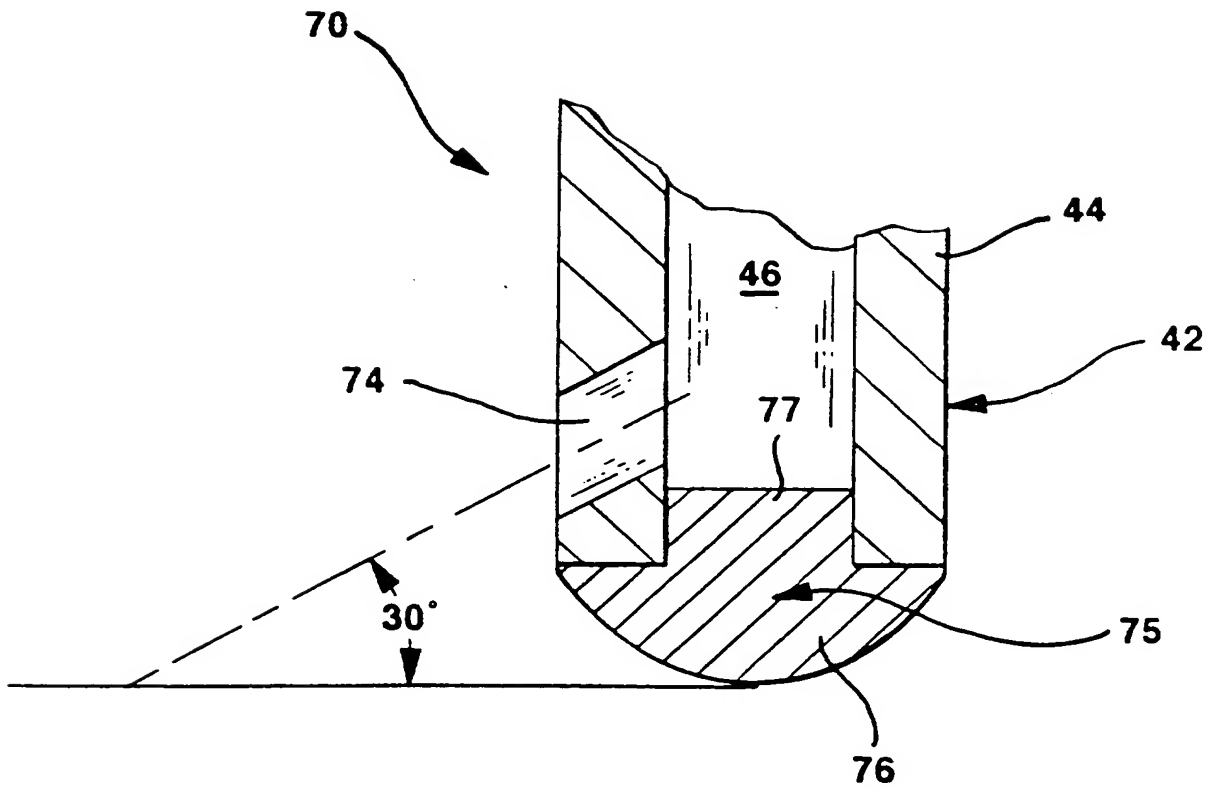

FIG.4

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US96/15132

A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) :G01N 35/10
US CL :Ple. e See Extra Sheet.
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 422/63, 100, 104; 436/43, 49, 54, 180; 73/864.01, 864.23; 134/157, 184

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	US 3,849,830 A (WAGNER) 26 November 1974, figure 2.	1-15
A	US 3,964,526 A (SINDERMANN) 22 June 1976, figure 2.	1-15
A	US 4,053,284 A (POSCH) 11 October 1977, whole document.	1-15
A	US 4,913,179 A (ENGEL et al.) 03 April 1990, whole document.	1-15

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

*	Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A"	document defining the general state of the art which is not considered to be of particular relevance	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"E"	earlier document published on or after the international filing date	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&"	document member of the same patent family
"O"	document referring to an oral disclosure, use, exhibition or other means		
"P"	document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search 23 DECEMBER 1996	Date of mailing of the international search report 11 FEB 1997
Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. (703) 305-3230	Authorized officer LONG V. LE Telephone No. (703) 308-0651

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US96/15132

A. CLASSIFICATION OF SUBJECT MATTER:
US CL :

422/63, 100, 104; 436/43, 49, 54, 180; 73/864.01, 864.23; 134/157, 184